Blueberry Microsatellite Markers Identify Cranberry Cultivars

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Abstract

Throughout the more than 100 years of cranberry, *Vaccinium macrocarpon* Ait., cultivation, the confirmation of genotype identity through morphological means has had inherent difficulties. The cranberry growth habit, where seedlings can grow in the middle of a clonal colony and the practice of taking clippings from old fields to establish new ones can destroy trueness-to-type. Previously, we successfully identified blueberry (*V. corymbosum* L.) cultivars using microsatellite markers. Our objective for this study was to determine if these markers could be used for cranberry cultivar identification. Forty-six blueberry simple sequence repeat markers (SSR), i.e., microsatellites, were tested for the ability to amplify a polymorphic marker in American cranberry accessions. Sixteen SSR resulted in informative and polymorphic primer pairs and were used to fingerprint 16 economically important cranberry cultivars. They distinguished between the cultivars and grouped them based on pedigree. Two accessions labeled as *V. macrocarpon* 'Searles', collected from Jacob Searles Cranberry Co. in Wisconsin, had different genetic profiles. They were differentiated from each other based on the proportion of shared allele distance using these SSR, thus demonstrating the power of these markers in identifying genetically different cranberry plants that share the same cultivar name. A subset of these 16 blueberry SSR markers will be used to fingerprint the cranberry collection of the US Department of Agriculture, Agricultural Research Service, National Clonal Germplasm Repository (NCGR), to evaluate genetic variation of important cultivars growing in Oregon and Washington fields and to provide web-access to these markers and fingerprints for the cranberry community.

INTRODUCTION

Cranberry belongs to genus *Vaccinium* L. section *Oxycoccus* which contains two species: *V. macrocarpon* Ait. and *V. oxycoccos* L. (Vander Kloet, 1983; Eck, 1990). *V. macrocarpon,* the American or large-fruited cranberry, is the commercial cranberry, an exclusively diploid ($2n = 2x = 24$) vine that is endemic to North America, with its natural distribution extending from Newfoundland, west throughout the Great Lakes Region to Minnesota, and south through the Appalachian Mountains to North Carolina and Tennessee. In contrast, *V. oxycoccos,* or little-leaved cranberry, is a polyploid species which has tetraploid ($2n = 4x = 48$), and hexaploid ($2n = 6x = 72$) individuals. *Vaccinium oxycoccos* is circumboreal.

Eck (1990) cites 132 cranberry cultivars that were selected from the wild from native North American bogs, and seven that were bred and released. At least a dozen cranberry cultivars, such as *V. macrocarpon* 'HyRed,' have been released since the early 1990s. The identity of cranberry cultivars is compromised by the vegetative spread of genetic variants originating from sexual propagation of volunteer seedlings and native clones in beds. The scarcity of qualitative morphological descriptors in cranberry also

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contributes to cultivar misclassification. Molecular markers offer a direct estimation of genetic diversity through a determination of the differences in the genetic material. Prior laboratory methods for identity determination include isozymes (Bruederle et al., 1996), Random Amplified Polymorphic DNA (RAPD) (Debnath, 2007; Novy et al., 1994, 1996; Novy and Vorsa, 1995; Polashock and Vorsa, 2002), and Sequence Characterized Amplified Regions (SCAR) (Polashock and Vorsa, 2002) markers. Lack of polymorphism has prevented the use of isozymes in cultivar identification of cranberry (Bruederle et al., 1996). The molecular markers used for fingerprinting and assessing genetic relatedness in cranberry cultivars have been limited to the simple but irreproducible RAPD method. Out of 22 cranberry cultivars analyzed using silver-stained RAPDs, only 14 cultivars had unique profiles and the remaining eight were represented by 3 profiles indicating cultivar misclassification (Novy et al., 1994). Fifty-two RAPDs were used to assess genetic heterogeneity and relatedness within accessions of the "Big Four" major cranberry cultivars 'Early Black', 'Howes', 'McFarlin', and 'Searles'. Each cultivar was represented by multiple genotypes, which in many cases did not appear related to each other (Novy et al., 1996). In a study aimed at characterizing the variability in production observed among 12 Washington 'McFarlin' bogs, a unique profile appeared to represent the true high-yielding original 'McFarlin' (Novy et al., 1996). The need for a more reliable and reproducible molecular marker prompted Polashock and Vorsa (2002) to develop SCAR markers from these polymorphic RAPDs. Nine SCAR primer pairs generated 38 easily scored markers in two multiplex reactions across 594 cranberry accessions. Comparison of RAPD and SCAR methods of assessing the genetic relationships among 27 cranberry genotypes resulted in variation in clustering and, thus, confusion in inferring relatedness. Attempts to automate SCAR analysis were limited by reduced marker intensity.

Microsatellites are now preferred because of their high information content, high reproducibility and codominant nature which enables genotype identification, and ease of use. The objective of this study was to evaluate 39 EST-SSR and 10 genomic SSR markers derived from *V. corymbosum* cv. Bluecrop for cross-transference into cranberry and for polymorphism in seven representatives of each cranberry type, *V. macrocarpon* and *V. oxycoccos*. Sixteen SSR that amplified in the American cranberry were further evaluated for their ability to distinguish 16 important cranberry cultivars.

MATERIALS AND METHODS

DNA was extracted from actively growing cranberry leaves in the spring using a modified protocol based on the Puregene kit (Gentra Systems, Inc, Minneapolis, Minnesota). Seven genotypes each of *V. macrocarpon* (PI618015, PI614076, PI614077, PI614078, PI555031, PI618059, PI618231) and *V. oxycoccos* (PI555193, PI555194, PI613707, PI613686, PI613698, PI618017, PI613681) present at the NCGR were used to test the 49 blueberry SSRs (Boches et al., 2005) for cross transference (Table 1). Sixteen SSRs that amplified in *V. macrocarpon* (Table 1) were used to identify 16 important cranberry cultivars (Table 2).

The optimum annealing temperature for each primer pair was determined by gradient PCR cycles in an Eppendorf Gradient thermocycler (Eppendorf, Westbury, New York) or an MJ Research (Watertown, Massachusetts) Tetrad thermocycler. PCR reactions were carried out in a total volume of 10 µl which contained 1X Biolase buffer, 2 mM $MgCl_2$, 200 µM each of dATP, dCTP, dGTP, and dTTP, 0.3 µM each of forward and reverse primers, 0.25 units of Biolase Taq DNA polymerase (Bioline Inc., Randolph, Massachusetts), and 2.5 ng of template DNA. The PCR program consisted of 35 cycles of 94°C for 40 s, optimum T_a for 40 s and 72°C for 40 s. A final extension at 72°C for 30 min was used to maximize non-templated adenosine addition to the 5' ends. For cross-transference into cranberry, amplification and polymorphism were scored after separating the PCR products by 3% agarose gel electrophoresis and DNA was visualized using a GelDoc 2000 (BioRad, Hercules, California) digital imaging system after ethidium bromide staining.

For distinguishing the 16 cranberry cultivars, fragment size estimation was determined after separating the PCR products by capillary electrophoresis using the Beckman CEQ 8000 genetic analyzer (Beckman Coulter, Inc., Fullerton, California).

PCR products amplified from sixteen SSR primer pairs were scored for presence or absence of each amplicon. The data were imported into PowerMarker (Liu and Muse, 2004) and used for neighbor joining cluster analysis (Fig. 1) based on the proportion of shared alleles distance.

RESULTS AND DISCUSSION

For both types of microsatellite markers, EST-SSRs and genomic SSRs, amplification and polymorphism were higher in V. *oxycoccos* as compared to *V. macrocarpon* leading to 29 potential markers in the little-leaf cranberry and to 23 possible markers in the American cranberry (Table 1). Of 39 blueberry EST-SSR loci examined, cross-transference was 82% in *V. macrocarpon* and 84.6% in *V. oxycoccos*. Polymorphism in the loci that cross-amplified in cranberry ranged from 46.2% in *V. macrocarpon* to 66.7% in *V. oxycoccos*. Of 10 genomic SSR loci examined, cross-transference was 60% in *V. macrocarpon* and 70% in *V. oxycoccos*. Polymorphism in the loci that cross-amplified in cranberry ranged from 83.3% in *V. macrocarpon* to 100% in *V. oxycoccos*. EST-SSRs generated a higher rate of amplification and a lower rate of polymorphism than those observed in genomic SSRs in both species as previously observed in other plants (reviewed by Varshney et al., 2005).

Seven SSRs (CA94, CA112, CA187, CA855, NA41, NA172 and VCC_B3) amplified up to two PCR products in each of the 16 diploid cranberry cultivars while the remaining SSRs generated up to seven amplicons indicating presence in multiple locations in the cranberry genome. Neighbor joining cluster analysis separated each cultivar and grouped them according to pedigree. For example, 'HyRed' grouped with its parents 'Stevens' and 'Ben Lear'. These SSRs were highly polymorphic in cranberry and generated different genetic profiles in two 'Searles' accessions collected from Jacob Searles Cranberry Co. in Wisconsin.

CONCLUSIONS

Microsatellite loci identified in blueberry were transferable to cranberry. Their usefulness will be assessed using a larger number of accessions and fragment analysis following capillary elecrophoresis. The establishment of base marker patterns for each cranberry cultivar will be useful for future comparisons of unknown to identified cultivars. This technique will allow for much closer determination of identity within particular cranberry fields, especially where trueness-to-type is questioned by observational morphological differences; or where suspect cultivar identity needs to be confirmed or unknowns identified.

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Tables

Table 1. Thirty-nine EST-SSRs (CA and NA prefix) and 10 genomic SSRs (VCC) from blueberry that amplified the expected size fragment. Their optimum annealing temperatures in cranberry as determined by gradient PCR, size range, amplification and polymorphism in 7 accessions of *V. macrocarpon* and in 7 accessions of *V. oxycoccos* are listed.

Locus	Ta (°C)	Size range	Amplification/polymorphism	
			V. macrocarpon	*V. oxycoccos*
CA23F	62	160-200	-/-	+/+
CA25F	54	220-280	-/+	+/+
CA61F	50	180-280	-/+	+/+
CA94F[1]	56	300-450	+/-	+/-
CA112F[1]	62	150-220	+/+	+/-
CA169F	62	110-140	+/+	+/-
CA187F[1]	54	120-200	+/-	+/+
CA190R	54	200-300	+/-	+/+
CA208F	56	210-260	+/-	+/-
CA214F	54	100-130	+/+	-/+
CA218F	65	120	-/-	+/-
CA222F	56	140	-/-	+/-
CA229F	56	180	+/-	+/-
CA236F	50	270-310	+/-	+/-
CA239F	-	-	-	-
CA278F	-	-	-	-
CA344F	54	150-250	+/-	-/-
CA421[1]	60	150-250	+/-	-/+
CA483F	54	280-340	+/-	-/+
CA518F	-	-	-	-
CA642F	50	310-410	-	-/+
CA664F	52	120-280	-/-	+/+
CA787F[1]	60	290-350	-/+	+/-
CA794F[1]	54	220-280	-/+	+/-
CA855F[1]	54	220-290	+/+	+/-
CA1031F	-	-	-	-
NA41[1]	51		+/-	+/+
NA137	54	190-200	+/-	-/-
NA172	62	300-360	+/-	-/+
NA222	-	-	-	-
NA240	54	90-120	-/-	+/-
NA247	52	310	-/-	+/-
NA295	64	130	-/-	+/-
NA398	56	200-270	-/+	+/+
NA741	-	-	-	-
NA800[1]	60	200-310	-/+	+/-
NA824[1]	60	180-220	-/+	+/-
NA961[1]	60	150-200	-/+	+/-
NA1040[1]	60	200-310	+/+	+/-
VCC1_B3[1]	62	220-310	+/+	+/+
VCC1_H9	-	-	-	-
VCC1_I2	-	-	-	-
VCC1_I8	-	-	-	-
VCC1_J1[1]	64	220-250	+/-	-/+
VCC1_J3[1]	58	150-200	+/-	-/+
VCC1_J5[1]	54	120-290	+/-	-/+
VCC1_J9[1]	62	220-330	+/-	-/+
VCC1_K4	54	190-300	-	+/+
VCC1_S10	60	150-300	-/-	+/+

[1]Sixteen SSRs were fluorescently labeled and used to distinguish 16 important cranberry cultivars.

Table 2. American cranberry cultivars that were fingerprinted using 16 SSR markers. The Plant Introduction (PI) number, NCGR local number, origin and pedigree are included.

Cultivar	PI no.	Local no.	Origin	Pedigree
Crowley	554976	111.001	Washington	McFarlin x Prolific
Beckwith	554990	496.001	New Jersey	McFarlin x Early Black
Ben Lear	554983	503.001	Wisconsin	Selected from the wild
Bergman	554982	662.001	New Jersey	Early Black x Searles
Prolific	554993	666.001	Michigan	Seedling selection
Early Black	554986	741.001	Massachusetts	Selected from the wild
Franklin	554998	743.001	New Jersey	Early Black x Howes
Searles	555013	775.002	Wisconsin	Selected from the wild
Searles	555013	775.003	Wisconsin	Selected from the wild
Stanley	618059	1046.001	Massachusetts	Katherine x Rubel
McFarlin	614075	1295.001	Massachusetts	Selected from the wild
Howes	614076	1296.001	Massachusetts	Selected from the wild
Pilgrim	614077	1297.001	New Jersey	Prolific x McFarlin
Stevens	614078	1298.001	New Jersey	McFarlin x Potter
Wilcox	614079	1299.001	New Jersey	Howes x Searles
HyRed			Wisconsin	Stevens x Ben Lear

Figures

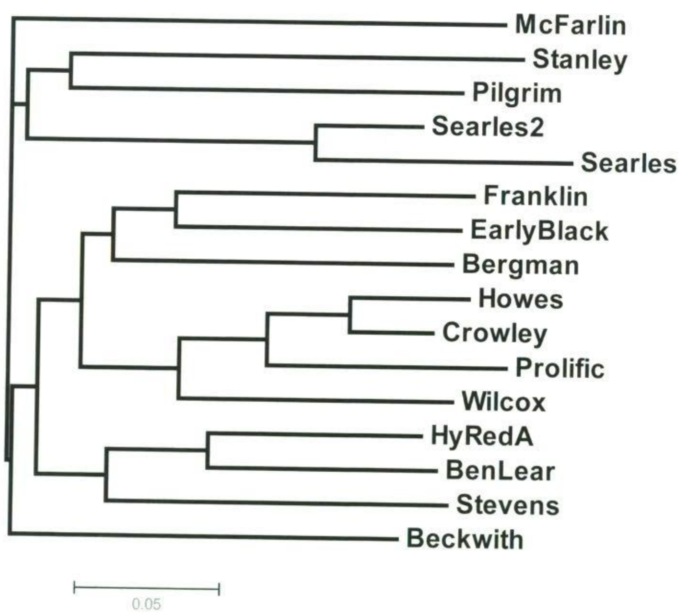

Fig. 1. Neighbor joining cluster analysis of sixteen American cranberry cultivars using the proportion of shared alleles distance based on 16 microsatellite markers.